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Procedure and Analysis of a Useful Method in Determining Mycelial Dry Weights from Agar Plates

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The evaluation of growth by dry weight determination of fungus mycelium for agar plates was examined. The data obtained were statistically analyzed. This method was shown to be sufficiently accurate to be used as an investigative tool.

The more widely used methods (1, 6) for monitoring the growth rate of fungi have been either mycelial dry weights obtained from liquid culture or the measurement of increased colony diameter. These methods have been particularly favored because they require neither elaborate equipment nor exhaustive assay techniques. However, there are numerous experiments in which the effect of certain parameters such as light do not lend themselves to this type of assay. One could not use liquid cultures in a shaker, as light spectra through flasks and moving liquid would be difficult to measure, and light effects on a spherical mycelial mass would not be uniform. Use of an increase in colony diameter on an agar plate would measure only lateral growth and neglect any differences in aerial growth.

Day and Hervey (3) tried to solve this problem by placing the fungus mycelium-agar content of a petri dish in hot water to achieve a separation and then filtered this through cheesecloth followed by different types of washes. They concluded that washing with hot water freed the mycelium of more agar than did cold water washing and that it most probably dissolved substances from the mycelium as well. Little statistical work has subsequently been done to date to provide a clear picture of the potentiality of this method in determining fungus dry weights. This paper includes an analysis of the feasibility as well as a detailed description of an agar assay method of determining mycelial dry weights which can be used as a criterion for evaluating fungal growth.

Based on past experiences with the dissolution of agar by boiling water, as well as the filtration rates and integrity of numerous filter papers, the following separation method was employed. The agar-mycelial contents of each petri dish tested in this experiment were removed and placed in a beaker of rapidly boiling distilled water for 4 min. The contents of the beaker were then filtered through a Buchner funnel by using predried (1 day at 70 C) and preweighed 9-cm Whatman no. 114 filter paper. Two 250-ml samples of rapidly boiling, distilled water were used to rinse the initial boiling flask and the resultant mycelial mat on the filter paper. A constant vacuum of 15 psi was maintained throughout the working procedure. After the final wash the sample was aspirated for 30 sec. Dry weights were obtained by placing the samples in a 70 C forced-air drying oven (Lab-Line) for 24 hr. The dry samples were then placed in a vacuum desiccator charged with Drierite desiccant (Drierite Co., Xenia, Ohio) for 6 days. A single-pan, rapid-reading balance (Mettler model H-10) equipped with desiccant was used to make all weighings. All accumulated data were subjected to a statistical analysis by computer employing the 07V program of the Biomedical Computer Programs (4). The program output included an analysis of variance (including the F ratio test for homologous variances) and the results of Duncan's multiple range test (5). The test fungus used in this experiment was *Diplodia zeae* (Schw.) Lev., one pathogen which causes stalk rot of corn in Illinois and other states (2).

Previously dried and weighed Whatman no. 114 filter paper was subjected to four treatments to establish the amount of standard deviation associated with the assay method. Five filter papers each were (i) redried and directly reweighed, (ii) handled by the assay

method but not subjected to any filtration stress, (iii) handled by the assay method and subjected to filtration stress with distilled water, and (iv) handled by the assay method with the filtration stress of a boiling agar solution. By including these four treatments, the sources of variation which might contribute to the resultant mycelial dry weights could be resolved. The amount of deviation attributed to the assay procedure is given in Tables 1 and 2. The analysis of variance shows that the F ratio is not significant, thus, the sample means are all taken from the same population (there was no difference between the results of the four treatments).

The cumulative effect of weighing and handling and the stress of water filtration was demonstrated by the third treatment. The variability found in this treatment may be considered to be the same as that found in the broth assay method because the procedures are the same. The last treatment shows the amount of variability inherent in the agar assay method. There is no statistical difference between the effects of the water and the agar assay methods on the resultant dry weights of the filter paper. Both assay methods may be expected to increase the apparent dry weight of a sample due to the retention of media components in the filter paper at a constant rate with a predictable amount of variability. The effect of hot water extraction on the mycelium would also be constant across an experimental design. Therefore, the mechanics of the agar plate-dry weight method are considered sufficiently sound to include its use as a tool for evaluating mycelial growth.

TABLE 1. *Deviation attributed to the agar assay method*

Treatment	Mean weight increase (mg)	Standard deviation
Reweighed immediately	7.500	1.09
Handled	6.720	3.04
Distilled water without agar	4.940	3.10
Boiling water with agar	4.020	2.98

TABLE 2. *Sources of variation in the agar assay method*

Source of variation	Sum of squares	Degrees of freedom	Mean square
Treatment	38.22	3	12.74
Replication	115.51	16	7.22
Total	153.73	19	

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